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Severe Liver Impairment in a Cystic Fibrosis-Affected Child Homozygous for the G542X Mutation

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The clinical and laboratory findings of a cystic fibrosis (CF) patient homozygous for the G542X mutation are described. This is the first case, among the 7 G542X homozygous CF subjects described so far who shows severe liver involvement, associated pancreatic insufficiency, and moderate pulmonary expression of the disease, as demonstrated by laboratory and imaging data. This case adds to the conclusion that genotype/phenotype correlation in cystic fibrosis is more complex than formerly suspected. Am. J. Med. Genet. 69:155–158, 1997. © 1997 Wiley-Liss, Inc.

KEY WORDS: cystic fibrosis; genotype/phenotype correlation; liver; G542X mutation

INTRODUCTION

Liver involvement has been described in 10–40% of cystic fibrosis (CF) patients [Colombo et al., 1994] and conflicting results have thus far been described concerning a possible role of genotype in the liver expression of CF; it has been also suggested that liver involvement in CF cannot be simply predicted by genetic markers [Colombo et al., 1994].

The G542X nonsense mutation [Kerem et al., 1990] is one of the most frequent CF mutations; in Campania and Basilicata (Southern Italy), the mutation has a frequency of about 5% on a sample of 274 recently analyzed cystic fibrosis (CF) chromosomes [Castaldo et al., 1996b], versus a frequency of 2.67% described for CF subjects from North Eastern Italy [Gasparini et al., 1993]. The G542X mutation may have been introduced into Mediterranean coastal areas by the Phoenicians and Arabs, and, in our particular area, also as a consequence of the Spanish domination of Southern Italy [Casals et al., 1993].

The few patients reported to be homozygous for the G542X mutation in the CFTR gene have heterogeneous clinical phenotypes, i.e., either a mild [Cuppens et al., 1993; Bonduelle et al., 1991; Beaudet et al., 1991] or a severe expression of the disease [Kalaydijeva et al., 1991; Bienvenu et al., 1993; Desgeorges et al., 1994]. None was reported to be affected by liver involvement. The patients with severe forms were affected early in life with characterized meconium ileus and early symptoms of pancreatic and lung disease (Table I). Here we describe a cystic fibrosis-affected child with a homozygous G542X mutation who showed, in addition to pancreatic and pulmonary involvement, severe liver expression of the disease.

CLINICAL REPORT AND METHODS

Clinical Report

All the procedures used in this study conformed to the ethical guidelines of the 1975 Declaration of Helsinki and agree with the guidelines of the Ethical Committee of our Medical School.

This CF-affected female, homozygous for the G542X mutation, died at the age of ten years. She was the youngest of two siblings born to healthy, unrelated parents originating from Southern Italy. She was born without meconium ileus and CF was diagnosed at the age of 4 months on the basis of failure to thrive, chronic diarrhea with steatorrhea and recurrent respiratory symptoms. The sweat test was positive (chloride = 98.6 mEq/L); since the age of 6 years the patient had

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TABLE I. Various Phenotypic Features in Seven Cystic Fibrosis G542X Homozygous Patients

Reference	Bonduelle 1991	Cuppens 1990	Beaudet 1991	Kalaydijeva 1991	Bienvenu 1993	Desgeorges 1994	Present case
Origin (country)	Belgium	Belgium	USA	Bulgaria	Turkey	France	Italy
Age of diagnosis	2 years	2 years	n.r.[a]	<1 year	5 months	5 months	4 months
Meconium ileus	No	No	No	No	Yes	No	No
Disease progression	Death at 12 years	Alive, 11 years	Alive, 19 years	Early death	n.r.	n.r.	Death at 10 years
Lung involvement	Moderate	Mild	Mild	Severe	Severe	Severe	Moderate
Liver involvement	No	No	No	No	No	No	Severe
Pancreatic insufficiency	No	No	Yes	n.r.	Yes	Yes	Yes
Weight (centile)	75–97th	75th	n.r.	n.r.	n.r.	n.r.	50th
Height (centile)	75–97th	90th	n.r.	n.r.	n.r.	n.r.	50th

[a]n.r., not reported.

lung colonization by *Pseudomonas aeruginosa* (presence of the bacteria in the sputum for more than 6 months).

Also at the age of 6 years, clinical, biochemical and ultrasonographic abnormalities indicated the presence of overt liver disease. The patient presented with severe hepato-splenomegaly and signs of portal hypertension and ascites. From the age of 3 years the patient showed concentrations of serum aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase and gamma-glutamyltransferase that were regularly more than twice the upper limit for her age. In addition, a decrease of serum albumin and total protein indicated a gradual impairment of protein synthesis activity. All other causes of chronic liver disease in children, i.e., metabolic, viral and genetic other than CF were ruled out by laboratory and historical data.

The clinical course was characterized by growth retardation with poor nutritional status, moderate pneumopathy (FEV1 = 53%, FVC = 55% and Chrispin-Norman score = 11 at the age of 9 years old), and progression of liver disease to liver failure with a severe decrease of prothrombin activity, serum protein and albumin, and increased portal hypertension with hypersplenism; ascites gradually became resistant to conventional therapy, and at the age of 10 years, the patient had several minor hemorrhagic episodes followed by an episode of severe hematemesis. Endoscopic examination showed grade III–IV esophageal varices. Clinical and ultrasound evaluations showed increased liver size with irregular surface, heterogeneity of liver parenchyma with gross dilation of the collateral venous circle; the biliary ducts appeared normal in size with absence of calculi. Histology showed fibrosis and multilobular biliary cirrhosis, which are typical findings of CF liver involvement [Colombo et al., 1994].

Nasal polyposis and diabetes did not appear during the course of the disease. The pancreatic status, as evaluated by physical examination and by fecal fat balance, indicated pancreatic insufficiency with minimal response to pancreatic-enzyme supplementation. The patient died at the age of 10 years from liver insufficiency.

Genetic analysis of the patient showed the G542X stop mutation in the homozygous state (Fig. 1); both parents carried the mutation. The XV 2c and KM 19 haplotypes associated with the G542X mutation were 1,1 and 2,2, respectively, as typically described for the G542X mutation [Casals et al., 1993]. The intron 8 VNTR haplotype was 23/23, the common allele associated with the G542X mutation [Casals et al., 1993]. Table II shows the laboratory evaluation of liver function at ages of 6 and 10 years. Liver function gradually deteriorated, particularly the protein synthesis activity, as shown by the severe decrease of serum albumin, cholinesterase, prothrombin activity (at a time when the patient was receiving a strong supplementation of vitamin K) and by the severe increase of ammonia. A significant reduction in circulating platelets was a sign of hypersplenism.

Molecular Analysis

The molecular analysis of CF mutations [De Marchi et al., 1994; Castaldo et al.,1996b] was performed with a semiautomated polymerase chain reaction (PCR) followed by allele specific oligonucleotide (ASO) dot blot using the Biomek 1000 workstation (Beckman) for both assembling the PCR mixtures and spotting the amplified DNA on dot blot filters (Hybond N^+, Amersham, UK). The extragenic XV 2c and KM 19 polymorphisms were analyzed using the Taq 1 and Pst 1 restriction enzymes, respectively [Rosenbloom et al., 1990; Feldman et al., 1988]. For both the polymorphisms, allele 1 corresponds to the absence of the restriction site and allele 2 to its presence. The intron 8 VNTR was analyzed by PCR amplification followed by polyacrylamide electrophoresis [Morral et al., 1991]; the haplotype numbering corresponds to the number of repeats.

DISCUSSION

Overt liver disease in CF can progress to multilobular biliary cirrhosis with portal hypertension and liver

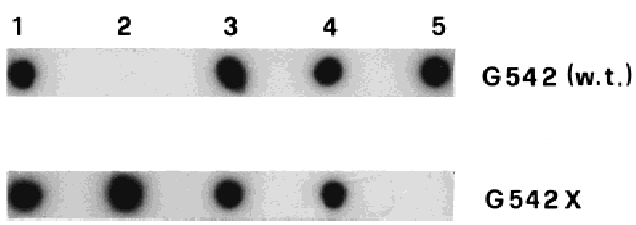

Fig. 1. Allele specific oligonucleotide (ASO) dot blot analysis of the CF G542X mutation. **Lane 1** = positive heterozygous control for G542X mutation; **lane 2** = patient: homozygous for the G542X; **lanes 3 and 4** = father and mother of the propositus, respectively; **lane 5** = negative, healthy control.

TABLE II. Laboratory Evaluation of the Patient at the Ages of 6 and 10 Years

Analyte	Evaluation at 6 years old	SI unit	Evaluation at 10 years old	SI unit	Reference intervals
Quick (activity)	79%		33%		80–100%
Cholinesterase	3700 U/L	61.68 μkat/L	877 U/L	14.62 μkat/L	2,800–7400 U/L
Albumin	4.1 g/dL	41 g/L	3.0 g/dL	30 g/L	3.2–4.5 g/dL
Ammonium	89 μg/dL	49.33 μmol/L	223 μg/dL	123.6 μmol/L	40–80 μg/dL
ALT	94 U/L	1.57 μkat/L	46 U/L	0.77 μkat/L	up to 49 U/L
AST	91 U/L	1.52 μkat/L	39 U/L	0.65 μkat/L	up to 35 U/L
AP	766 U/L	12.77 μkat/L	991 U/L	16.52 μkat/L	300–660 U/L
GGT	109 U/L	1.82 μkat/L	176 U/L	2.94 μkat/L	up to 14 U/L
Bilirubin	1.21 mg/dL	20.69 μmol/L	2.10 mg/dL	35.91 μmol/L	0.20–1.00 mg/dL
Hemoglobin	11.7 g/dL	117 g/L	7.2 g/dL	72 g/L	11.5–14.0 g/dL
Platelets	136,000/mm^3		78,000/mm^3		120,000–400,000/mm^3

failure [Roy et al., 1982]. Genetic predisposition for CF liver involvement has been postulated on the basis of familial concordance [Duthie et al., 1992; The Cystic Fibrosis Genotype-phenotype Consortium, 1993]. However, a recent study failed to demonstrate a specific correlation between genotypes and the development of hepatopathy in Italian CF patients [Colombo et al., 1994]. The case we describe seems to demonstrate that the CF mutation is a genetic factor necessary but not sufficient to completely predict the liver expression of CF. Other environmental or genetic factors could influence the expression of the liver disease.

The clinical course of CF can be influenced by environmental, epigenetic and therapeutic factors, but the mechanisms responsible for the heterogeneity of phenotypes are still unclear. The G542X mutation in the homozygous state may be associated with clinically different degrees of pancreatic and pulmonary involvement (see Table I). In homozygous ΔF508 CF patients, the phenotype is affected by ethnic background as well as by the genotype [Lester et al., 1994]. It is conceivable that the phenotypic heterogeneity arising from the G542X mutation could also be influenced by the different ethnic background of the patients so far described (see Table I). It is noteworthy that in our population of 137 CF patients from Campania and Basilicata (Southern Italy) analyzed thus far, the G542X mutation was detected in 15 out of 274 chromosomes (5.4%), and two compound heterozygous genotypes were found among G542X CF bearing patients, i.e., G542X/ΔF508 in 9 cases and the G542X/unknown genotype in 4. All 13 CF patients were affected by pancreatic insufficiency, but none by liver involvement, except for a borderline increase of alkaline phosphatase (<1.5 times the upper limit) in three cases.

Four of the 7 G542X homozygous CF patients described to date had only mild or moderate pulmonary expression, which is surprising given the nonsense nature of the G542X mutation and the fact that the G542X mutation results in a very low level of mRNA transcript [Hamosh et al., 1992]; we have recently obtained similar results in a homozygous patient bearing the R553X mutation, which also is a nonsense mutation [Castaldo et al., 1996a]. As previously suggested [Bienvenu et al., 1993], alternative pathways might be operative in some tissues.

The diverse expression of the disease in the above 7 patients again casts doubt on the possibility to predict straightforward genotype/phenotype correlations. Hopefully, specific mRNA analysis, possibly in different tissues from the same patient, will lead to a better understanding of the different clinical findings in individuals affected by the same disease.

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